APR 25 1931

Volume XIII MARCH, 1931 Number 3

THE JOURNAL OF INDUSTRIAL HYGIENE

WITH ABSTRACT OF THE LITERATURE

Editor, United States
DAVID L. EDSALL, M.D., S.D.

Honorary Consulting Editor
SIR THOMAS M. LEGGE, M.D., D.P.H.

Editor, Great Britain
E. L. COLLIS, M.D., M.R.C.S.

A Method of Staining the Asbestosis Bodies Found in the Sputum of Asbestos Workers

The Toxicity of Certain Benzene Derivatives and Related Compounds

Studies on Experimental Pneumonokoniosis. VI. Inhalation of Asbestos Dust: Its Effect upon Primary Tuberculous Infection
(Concluded)

Published at Mount Royal and Guilford Aves., Baltimore, Md.
by HARVARD SCHOOL OF PUBLIC HEALTH

Entered as second-class matter, at the Post Office at Baltimore, Maryland, under Act of March 3, 1879.
Copyright, 1931, by Harvard Medical School

Made in the United States of America

EDITORS

United States
DAVID L. EDSALL, M.D., S.D.

Great Britain
E. L. COLLIS, M.D., M.R.C.S.

HONORARY CONSULTING EDITOR
SIR THOMAS M. LEGGE, M.D., D.P.H.

ASSOCIATE EDITORS

United States

W. IRVING CLARK, JR., M.D.
LELAND E. COFER, M.D.
ALICE HAMILTON, A.M., M.D.
EMERY R. HAYHURST, A.M., Ph.D., M.D.
YANDELL HENDERSON, Ph.D.
HARRY E. MOCK, M.D.
L. R. THOMPSON, M.D.
C.-E. A. WINSLOW, M.S., A.M., Dr.P.H.
WILLIAM P. YANT, B.S.

Great Britain

W. F. DEARDEN, M.R.C.S., D.P.H.
SIR KENNETH GOADBY, K.B.E., M.R.C.S., D.P.H.
SIR LEONARD HILL, M.B., F.R.S.
T. LISTER LLEWELLYN, B.S., M.D., M.I.M.E.
SIR THOMAS OLIVER, M.D.
R. PROSSER WHITE, M.D., M.R.C.S.
H. M. VERNON, A.M., M.D.
D. A. COLES, M.D.

South Africa

E. H. CLUVER, A.M., M.D., B.Ch., D.P.H.

Canada

J. J. R. MACLEOD, M.B.

Australia

CHARLES BADHAM, M.D.

MANAGING EDITORS

CECIL K. DRINKER, M.D.
PHILIP DRINKER, B.S., Ch.E.
MARION C. SHORLEY

THE JOURNAL OF INDUSTRIAL HYGIENE

VOLUME XIII MARCH, 1931 NUMBER 3

A METHOD OF STAINING THE ASBESTOSIS BODIES FOUND IN THE SPUTUM OF ASBESTOS WORKERS*

S. ROODHOUSE GLOYNE, M.D., D.P.H.

Pathologist, City of London Hospital for Diseases of the Heart and Lungs, Victoria Park, London

THERE is a consensus of opinion that the asbestosis bodies found in the sputum and lungs of asbestos workers do not stain with the ordinary aniline dyes. This failure to stain has been one of the difficulties in working out their relation to the histology of the disease and in determining their composition. By employing the following technic the bodies can be made to take on a definite color which enables them to be better studied.

HEMATOXYLIN

After digestion of the sputum with equal quantities of concentrated antiformin (as described by Stewart and Haddow (1)) and centrifugation, the antiformin is pipetted off and replaced by a 5 per cent. solution of Ehrlich's hematoxylin. Bluing of the hematoxylin immediately takes place owing to the remains of the alkaline antiformin. The mixture is well shaken and allowed to stand for a half to one hour and then recentrifuged and the deposit mounted as a wet preparation. By this means the asbestosis bodies become a dark brown to black color, according to the length of time they have been exposed to the hematoxylin.

PRUSSIAN BLUE REACTION

The reaction of the asbestosis body to Prussian blue was first described by Cooke and Hill (2) and McDonald (3). The foregoing technic is used but the following mixture is substituted for hematoxylin: 2 per cent. potassium ferrocyanide—1 part; 1 per cent. hydrochloric acid—3 parts. With this technic the asbestosis bodies are colored a brilliant blue. A potassium ferricyanide mixture may also be used but the results are variable, some asbestosis bodies being stained, others not.

AMMONIUM SULPHIDE

The same technic is used but ammonium sulphide is substituted for hematoxylin or potassium ferrocyanide. The asbestosis bodies are then colored

* Received for publication Nov. 22, 1930.

black. This color can be removed with hydrochloric acid.

Discussion

When examined microscopically the hematoxylin specimens give the impression of having hematoxylin deposited on them rather than having actually taken up the stain, but after washing overnight the asbestosis bodies still retain sufficient stain to be colored a dark brown. With the ferrocyanide or ammonium sulphide technic the bodies are homogenously stained.

The advantages of this technic are: (1) The minute details of the asbestosis body can be better seen than in the unstained preparation; *e.g.*, the central core of asbestos fiber generally stands out clearly and can sometimes be seen running across a gap between two segments of the asbestosis body rather like the string between beads on a necklace. The fiber takes on the stain only very lightly—sometimes, indeed, not at all—but the contrast with the stained body is sufficient to demonstrate quite clearly its existence. The outlines of the segments are also very clearly seen, and the small subsidiary bosses on the sides of the asbestosis body, and the cracks in the segments are better distinguished by this method than in the unstained preparation. (2) These reactions support the view that iron enters into the composition of the bodies. The Prussian blue reaction is a well-known test for iron; and ammonium sulphide is universally employed as a group reagent for iron. Hematoxylin also has an affinity for iron, but the reaction in this case is inferior to that given by the other methods.

The amount of iron found in raw asbestos varies considerably. According to Merewether (4) the two main varieties of asbestos used in industry are: (1) a larger portion known as chrysotile, a hydrated magnesium silicate with a low percentage of iron oxides (0.7 to 4.4 per cent.); and (2) a smaller portion known as crocidolite, amosite, and tremolite which have a high percentage of iron oxides (3.2 to 44 per cent.). The latter group is the one used in the factory which produces the cases seen at this Hospital.

Cooke and Hill (5) have also suggested that blood may enter into the composition of the asbestosis body. Oxyhemoglobin contains 0.335 per cent. of iron (6).

BIBLIOGRAPHY

1. Stewart, M. J., and Haddow, A. C.: Demonstration of the Peculiar Bodies of Pulmonary Asbestosis ("Asbestosis Bodies") in Material Obtained by Lung Puncture and in the Sputum. Jour. Path. and Bacteriol., 1929, *32*, 172.
2. Cooke, W. E., and Hill, C. F.: Pneumokoniosis Due to Asbestos Dust. Jour. Roy. Micr. Soc., 1927, *47*, 232.
3. McDonald, S.: Histology of Pulmonary Asbestosis. Brit. Med. Jour., 1927, *2*, 1025.
4. Merewether, E. R. A.: The Occurrence of Pulmonary Fibrosis and Other Pulmonary Affections in Asbestos Workers. This Jour., 1930, *12*, 198, 239.
5. Cooke, W. E., and Hill, C. F.: Further Observations on Pulmonary Asbestosis, with Special Reference to Asbestos Dust and the Curious Bodies Found in the Lungs. Jour. Roy. Micr. Soc., 1930, *50*, 15.
6. Starling, E. H.: Principles of Human Physiology. Fourth edition. London, J. & A. Churchill, 1926.

J. I. H.
March, 1931

THE TOXICITY OF CERTAIN BENZENE DERIVATIVES AND RELATED COMPOUNDS*

HENRY FIELD SMYTH, M.D., DR.P.H.

Assistant Professor of Industrial Hygiene, University of Pennsylvania

INTRODUCTION

THE title of this paper may suggest a very wide range of compounds, about the toxicity of some of which we have considerable information but about that of others we know little or nothing. I was recently appealed to by an insurance company to outline a program for safe handling of a compound that I did not know was used in industry and about the toxicity of which nothing has been published so far as I know.

However, though the derivatives of benzene are legion, yet they group themselves into certain main classes, about certain members of which we have information. In most cases members of a class act on the system in a similar manner and differences in action are differences of degree rather than of kind. There are exceptions to this general rule, however, some of which will be pointed out later.

GENERAL CONDITIONS

Benzene, C_6H_6, is the simplest compound of all, and the benzene ring is the basis on which all the others are formed. The so-called homologues of benzene are toluene, $C_6H_5 \cdot CH_3$, and xylene, $C_6H_4 \cdot (CH_3)_2$, in which one or two methyl radicals replace one or two hydrogens (1).

The most important benzene derivatives industrially and toxicologically are the nitro, amino, diamino, and chlor compounds of benzene and toluene. Danger to health from these compounds depends not only on their chemical structure but also largely on their physical state and the method of handling them. Those compounds responsible for the greatest amount of industrial sickness are not necessarily the most toxic.

Homologues of Benzene

Considering first the homologues of benzene, toluene and xylene, we find somewhat conflicting reports. Selma Meyer (2) states that regardless of which poison is acting, whether benzene, toluene, xylene, naphthalene, aromatic nitro compounds, etc., the lymphocytes are increased in the blood stream and the neutrophils are forced back or repressed. American findings do not agree with this. Neither Greenburg (3) nor we (4) have found toluene or xylene presenting any characteristic blood picture in experimental animals. Greenburg found that with continuous exposures for up to seven days, high concentra-

* Read before the Section on Industrial Hygiene of the American Public Health Association, Fort Worth, Texas, Oct. 28, 1930.

Received for publication Dec. 15, 1930.

tions of toluene killed all exposed animals in forty-eight to seventy-two hours with narcosis. Animals surviving lower concentrations showed evidences of narcosis, irritation or depression, with irritability. Our own tests with four-hour exposures over longer periods showed evidences of lung inflammation and of toxic degeneration in internal organs. We both found toluene to be less toxic than benzene, and the National Safety Council recommended it as a substitute for benzene where possible (3). In addition to being less toxic, it is less volatile and is less likely to exist in a plant as vapor of toxic concentration. Xylene we have found also to be quite toxic, but again it is even less volatile than toluene and therefore less hazardous. Neither of these two substances seems to be readily absorbed through the skin in toxic amounts and I know of no case of poisoning from skin absorption due to them.

Nitro and Amino Group Compounds

Nitro and amino groups, aromatics, in general produce much the same clinical pictures, differing in some details and with a few striking exceptions. The latter in general are simple blood poisons, while the former in addition exert a direct action on the central nervous system. Floret (5) says that all aromatic and aliphatic compounds are fat solvents and therefore particularly apt to affect the central nervous system.

In light cases of poisoning from nitro or amino compounds there is flushing of the face, with a sense of fulness and throbbing pain in the head, burning sensation in the throat, and a feeling of tightness in the chest. More marked cases of poisoning develop violent throbbing headache, dizziness, roaring in the ears, and visual disturbances. With still more severe poisoning the face becomes livid, the lips and tongue blue, the knees weak, and the gait staggering. The blue color of the face may persist for several days. In extreme cases cyanosis increases, muscular tremors develop, there is extreme weakness, cold skin, nausea and vomiting, abdominal cramps, quick, shallow respiration, lowered blood pressure, and late unconsciousness. Coma develops, the respirations become progressively slower and shallower, involuntary urination and defecation may occur, and convulsions usually come on just before death. Not infrequently attacks are delayed, coma coming on even hours after cessation of exposure.

The blood picture is characteristic, with reduction in the red cell count and the hemoglobin, poikilocytosis, anisocytosis, and some fragmentation of red cells with polychromatophilia. The blood becomes chocolate colored owing to the development of methemoglobin, the spectroscope showing absorption bands between those of pure methemoglobin and oxyhemoglobin. Evidences of blood regeneration are seen after a few days in non-fatal cases, with the appearance of stippled cells and nucleated red cells. There is an early polymorphocytosis followed later by a relative lymphocytosis. In slow poisoning from continued low exposures there may be an increase in the red cell count. The urine becomes brown, port wine colored, or smoky red, and shows bile and blood pigments, methemoglobin or hemoglobin. At times albuminuria

occurs and the urine may reduce Fehling's solution. For most of this group the skin is the most important entry port.

Amino Compounds

While the amino compounds as a class produce a deeper cyanosis than do the nitro compounds, yet with them, poisoning is less serious and recovery usually results in a few days if exposure is not continued. There are exceptions to this, however. They are all very readily absorbed through the skin as well as through the lungs.

Aniline poisoning is first manifested as an intense cyanosis, the victims of poisoning being referred to as "blue boys." In an aniline plant visited during the war, cases of poisoning occurred daily and blue boys were a common sight. Following cyanosis there develop headache, dizziness, dysphagia, nausea, vomiting, weakness, restlessness, palpitation, and irregular slow respirations with rapid feeble heart action. Pupils are contracted but respond to light. Temperature is subnormal. There is an aniline odor on the breath and to the sweat. The urine is dark in color owing to the presence of hemoglobin. In severe cases there may be a loss of sphincter controls and also pulmonary edema. More cases and more severe poisonings occur in hot weather. Workers may develop a degree of tolerance but the cyanosis may persist. Continued exposure may result in headaches, irritability, poor appetite, neurasthenia, visual disturbances and itching of the eyes which may lead to injury and ulceration of the cornea from rubbing. There is anemia, with decreased hemoglobin. Long-continued exposure is said to produce bladder tumors which may become malignant.

The average lethal dose is 25 gm.; 0.4 to 0.6 mg. per liter of air may be borne without much harm for one-half to one hour but 0.1 to 0.25 mg. per liter for several hours produces slight symptoms (6). Tests made by Iszard (7), in 1920, in the aniline house of a plant while reducers were being discharged, gave the following amounts in milligrams per liter of air: 0.5, 1.01, 1.18, 2.43, and 3.4—all but the first one well above the toxic concentrations. Men were exposed to these concentrations for periods of from twenty to forty minutes. One workman at the plant developed cyanosis from wearing an old pair of gloves found lying in a pool of aniline water on a drumhead. Changes in design of apparatus and method relieved this condition and reduced the number of poisonings in this department almost to the vanishing point; later, however, the plant started to develop the production of synthetic indigo, and new cases began to appear in men discharging the phenylglycine driers, as the finely powdered phenylglycine carried more or less absorbed aniline. Here the poisoning was due to dust inhalation. Two tests taken by Iszard, in the indigo house while the vacuum driers were being emptied, showed 0.7 and 1.7 mg. of aniline per liter of air.

Toluidines produce the same symptoms as does aniline, with less cyanosis but more strangury and hemoglobinuria. They produce subnormal temperature and anemia. Exposure occurs from splashes from centrifuges and whizzers.

Beta-naphthylamine produces cyanosis and also frequent urination as a result of irritation due to overacid urine.

Diamines

The diamines may be decidedly toxic. Phenylenediamines are used as dye intermediates in dyeing hair and furs. They have been responsible for a number of cases of poisoning from hair dyes and from cheap dyed furs. Symptoms produced are dermatitis, sleeplessness, dizziness, and weakness; and epileptiform convulsions, coma, and death may result. The reaction may develop very suddenly and be of anaphylactic type. Some writers have insisted that it was a true anaphylaxis, and Curschmann has suggested calcium therapy with the inhalation of sprayed solutions of calcium salts. Most writers, however, now attribute the poisoning to quinone intermediates resulting from incomplete oxidation, and state that care to secure complete oxidation and careful, thorough washing of dyed furs will prevent the trouble from developing. Phenylenediamine should never be used in hair dyes.

Tolylenediamine is a blood poison, producing destruction of red cells, methemoglobin, and toxic jaundice with liver degeneration, but is less toxic than the phenyl salt.

Animal Experiments

At various times in the past few years our laboratory has been called upon to determine the toxicity of a number of benzene derivatives and allied substances, more or less complex, about whose effects on the system there was little or no information available in the literature. Many of these preparations were for use in the rubber industry as accelerators or retarders. Most of these tests have been assembled for comparative purposes and are here presented in the hope that they may be of interest and of help to others called upon to use these materials or to make similar tests.

In many cases all that was desired by those requesting information was the determination of the minimum lethal dose, as an indication of the possibility of accidental acute poisoning. With others the effects of repeated sublethal doses were studied.

All experimental work was done with small animals, it being the custom in our laboratory to use at least two types of animal, often three, for such work. The animals of choice, for convenience in handling, are white rats, guinea-pigs, and rabbits. Dosages are always given as grams per kilo of body weight of the animal at the time of administration. It is customary to interpret the results of such tests in terms of grams per kilo of body weight of man, but we always give such interpretation with considerable reserve, and feel that at best it indicates an approximation to the range of toxicity for man, rather than an actual measurement of it. When used for comparison of the toxicity of different compounds, however, we feel that such tests are a very fair indication of relative harmfulness.

When death occurs within a comparatively few hours from a single dose of a chemical, we rarely find microscopic evidence of toxic organ degeneration, and in these cases we report only dose, time of death, symptoms, and

gross changes noted at autopsy. When animals survive more than twenty-four hours, in addition to gross changes we always look for histologic changes in internal organs, especially the liver, kidney and spleen, and other organs as indicated. When animals survive a week or more, we usually include urine and often blood findings in the reports.

With most of the tests here reported on for minimum lethal dose determinations, no microscopic findings are included. The tests to be reported were made as parts of six different investigations on materials supplied by three different industrial firms. Firm names and trade names of chemicals or compounds are purposely omitted, but empirical and line structural formulas are given when known. In some instances, however, there are differences of opinion as to the structural formula of the compounds reported upon. When materials to be tested were water soluble, they were fed by pipette in watery solution. When not water soluble, if soluble in dilute acid they were fed as hydrochlorides with as little as possible excess acid. Liquids not mixable with water were fed as a starch paste. Solids insoluble in water or dilute acid were fed as a paste in olive oil or water. In all cases, animals being dosed were held in the hand of the operator and it was made sure by personal observation that all the material was actually swallowed. Except as noted, all materials were of the technical grade of purity.

Guanidine and Guanidine Derivatives

Guanidine, CH_5N_3 or $(NH_2)_2 \cdot C{:}NH$, is not in itself a benzene derivative but it enters into compounds that are. The minimum fatal dose for a pure sample of this substance was found to be 0.3 gm. per kilo when dissolved in a weak acid solution and fed as a hydrochloride. With this dose, death occurred in from three to twelve hours, preceded by twitchings, convulsions, collapse, and gasping inspirations.

Diphenylguanidine, $C_{13}H_{13}N_3$ or $(C_6H_5NH)_2 \cdot C{:}NH$, with two substituted phenyl radicals, also fed as a hydrochloride, killed in from one to three hours in a dose of 0.25 gm. per kilo, with the same train of symptoms.

Triphenylguanidine, $C_{19}H_{17}N_3$ or $(C_6H_5NH)_2 \cdot C{:}NC_6H_5$, with three phenyl radicals, fed as a paste in 0.1 per cent. hydrochloric acid, as it would not go completely into solution, killed in from three to four hours in a dose of 0.35 gm. per kilo; death was preceded by violent trembling, convulsions, gasping, violent chewing motions, and characteristic rolling over, always in the same direction.

The addition of the phenyl radicals to the guanidine molecule definitely increased toxicity. The apparent lessening of toxicity of the tri over the di product was no doubt due to its lessened solubility; even though it required a larger dose to kill, the prelethal symptoms were more violent.

Diorthotolylguanidine, $C_{15}H_{17}N_3$ or $(C_6H_4CH_3NH)_2 \cdot C{:}NH$, killed in forty-five minutes in a dose of 0.12 gm. per kilo; this also was fed as a hydrochloride in solution. Prostration developed rapidly, with twitchings, irregular shallow breathing, gasping, and cyanosis. Larger doses killed in twelve minutes. Here the tolyl radical produced a marked increase in toxicity. That the tolyl radical does not always insure toxicity is seen in the case of diorthotolylthiourea, $C_{15}H_{16}N_2S$

or $(C_6H_4CH_3NH)_2 \cdot CS$, in which it is combined with a harmless sulphur compound. Here no symptoms were observed with a dose of 4 gm. per kilo, though the same tendency, in a slight degree, was seen to the development of lung hemorrhage as was observed in almost all of these series. Dosages were not increased beyond 4 gm. per kilo because the corresponding dose for man would be tremendous and it was extremely difficult to feed larger doses to animals.

With the materials of the guanidine series there was a very sharp line between the fatal dose killing in at most a few hours and the dose from which there was apparent complete recovery. No animal that survived overnight died. The behavior of the animals dying from these materials strongly suggested cyanide poisoning, as did the behavior of one factory employee whose death was possibly the result of an accidental overdose of one of the guanidine derivatives.

A series of animals were fed sublethal doses daily of diorthotolylguanidine for up to twelve days. In these animals traces of a substance reducing Fehling's solution appeared in the urine after each feeding, but did not persist overnight. They all developed mild albuminuria but no casts, and postmortem examination showed early but not marked liver and kidney changes. There was evidently no rapid cumulative effect.

Aniline and Aniline Derivatives and Compounds

A number of aniline derivatives were tested and compared with aniline. Aniline itself, C_6H_7N or $C_6H_5NH_2$, reported in the literature as fatal for man in doses of 0.35 to 1.43 gm. per kilo (as a pure preparation), killed guinea-pigs in doses of 1.75 gm. per kilo when fed as pure aniline

Thiocarbanilide, $C_{13}H_{12}N_2S$ or $(C_6H_5NH)_2 \cdot CS$, a thiourea derivative, proved nonfatal in doses of 4 gm. per kilo, fed as a paste in olive oil, though the animals seemed weak and depressed and petechiae were seen in the lungs. Lack of solubility lessened toxicity, as did also the entrance of sulphur.

Methylenedianilide, $C_{13}H_{14}N_2$ or $(C_6H_5NH)_2 \cdot CH_2$, was also nonfatal in doses of 4 gm. per kilo, and no symptoms were observed. This material also was fed as a paste in olive oil, and insolubility probably was the reason for nontoxicity.

Paranitrosodimethylanilide, $C_8H_8N_2O$ or $NO \cdot C_6H_4N(CH_2)_2$, a nitroso compound fed as a paste in water, proved to be twice as toxic as aniline, even though not easily dissolved. It was fatal in doses of 0.65 gm. per kilo in from twelve to forty-eight hours, death being preceded by prostration and convulsions, and red staining of the urine.

A series of condensation products containing aniline gave interesting results. Formaniline $(C_7H_7N)_x$ or $(C_6H_5N:CH_2)_x$, a combination of two definitely toxic substances but itself soluble with difficulty was not fatal in doses of 4 gm. per kilo.

A trade compound, a condensation product of aniline, acetaldehyde and formaldehyde, and another made from aniline, acetaldehyde and carbon disulphide, also very slightly soluble and fed as olive oil pastes, were not fatal in doses of 4 gm. per kilo.

Paratoluidine, C_7H_9N or $CH_3 \cdot C_6H_4 \cdot$

NH_2, (pure preparation) fed as a paste in water, killed in a dose of 1.1 gm. per kilo. This was surprising as Hamilton (1) quotes Gibbs and Hare as giving 0.1 gm. per kilo as fatal for animals.

Anhydroformaldehyde-paratoluidine, $(C_8H_9N)_x$ or $(CH_3 \cdot C_6H_4N : CH_2)_x$, fed as a paste in 0.1 per cent. hydrochloric acid, was less toxic, the fatal dose being 2 gm. per kilo, killing in twelve hours; death was preceded by trembling and kicking, but no general convulsions occurred. This is the toluidine analogue of formaniline reported previously, and the substitution of toluidine for aniline markedly increases toxicity.

A condensation product of aniline, paratoluidine, butyraldehyde and carbon bisulphide was more toxic than anhydroformaldehyde-paratoluidine, but not so toxic as paratoluidine. It killed in doses of 1.7 gm. per kilo in from two to six hours; death was preceded by prostration and collapse but there were no convulsions. This was an oily fluid with presumably a smaller molecule than anhydroformaldehyde-paratoluidine, and size of molecule seems to bear some inverse ratio to toxicity.

Diphenyl and Diphenyl Derivatives

Technical grade diphenyl, $C_{12}H_{10}$ or $C_6H_5 \cdot C_6H_5$, prepared by passing benzene vapor through a red-hot iron tube, little if at all soluble in water but readily soluble in alcohol and ether (8), was not toxic in doses of 4 gm. per kilo.

Two chlorodiphenyl preparations, $C_{12}H_9Cl$ or $C_6H_5 \cdot C_6H_4Cl$, were tested and both proved slightly toxic; the 2-chloro compound killed in from twenty to forty hours in a dose of 2.5 gm. per kilo, with mild convulsions, and the 4-chloro compound killed in forty hours in a dose of 3.5 gm. per kilo, with no convulsions. Both were fed in pastes as they were apparently insoluble. Here the position of the chlorine atom seemed to govern the degree of toxicity.

Two polychlorodiphenyls, the definite compositions of which were undecided, proved nontoxic in doses of 4 gm. per kilo. They were also fed in pastes, and were composed of even larger molecules than the monochloro compounds.

A 4-nitrodiphenyl, $C_{12}H_9NO_2$ or $C_6H_5 \cdot C_6H_4NO_2$, in spite of its nitration was not fatal in doses of 4 gm. per kilo, owing probably to its insolubility.

Nitrobenzene

Nitrobenzene, $C_6H_5 \cdot NO_2$, decidedly toxic as a vapor and by skin absorption, the latter due to its solubility in fat, was less toxic (using a pure preparation) than we had anticipated, when fed in a starch paste, the fatal dose being 1 gm. per kilo for animals.

Phenylnaphthylamines

Both alpha and beta naphthylamine, $C_{10}H_9N$ or $C_{10}H_7 \cdot NH_2$, are decidedly toxic but their phenylated compounds, $C_{16}H_{13}N$ or $C_{10}H_7 \cdot NH \cdot C_6H_5$, proved very little toxic. The alpha compound fed as a paste required 4 gm. per kilo to kill in three days, while the same dose of the beta compound was not fatal. Here the alpha position proved the more toxic.

Tolylenediamines

We have stated above that tolylenediamine is a blood poison, producing

destruction of red cells, methemoglobin, toxic jaundice, and liver degeneration. It has, however, been suggested as a substitute for the phenylenediamines as a hair dye, on the ground of its much lower toxicity.

Tests were made with both the para and the meta compounds, $C_7H_{10}N_2$ or $CH_3 \cdot C_6H_3 \cdot (NH_2)_2$, the former as an ingredient of a hair dye and the latter as a rubber chemical.

Metatolylenediamine was fed as a hydrochloride in water. The fatal dose was 3 gm. per kilo; however, 0.7 gm. per kilo daily for nineteen days was fatal to a guinea-pig, and 0.6 gm. per kilo daily for five days killed a rabbit. In animals surviving several days there was evident definite fatty degeneration and also renal degeneration. The body fluids were stained with the material and chemical tests showed that some of it was excreted unchanged in the urine. When combined with unvulcanized rubber it was not extracted from the rubber when that was applied to the skin and held there as a poultice for several hours, though it was very slowly extracted by a buffered watery solution ranging between a hydrogen ion concentration of 3 and of 7. The toxicity tests for this product seem to agree fairly well with those previously reported by Stadelmann (9) though he did not report in grams per kilo.

Paratolylenediamine was tested as an ingredient of a hair dye. For testing, this material was used in solutions of three different strengths as recommended for dyeing, containing amounts of the active ingredients ranging from 2 to 39.6 per cent., depending on the depth of color desired. The minimum fatal dose by mouth was found to be about 3.6 gm. of the pure substance per kilo—not very toxic. We found the material toxic to this extent by mouth and by subcutaneous injection, but could see no evidence of absorption through the unbroken skin. When used as a dye it is oxidized with hydrogen peroxide to bring out the full depth of color.

General Pathology of Entire Series

The one outstanding pathologic lesion seen in animals of the series poisoned with the various benzene derivatives was a tendency to extravasation of blood in the lungs, whether the animal died from the drug or was killed for study. This varied from scattered minute petechiae to larger ecchymoses or even massive lobular or lobar hemorrhage, the severity of the lesion usually paralleling the severity of the poisoning. In order to avoid agonal congestions seen in gassed animals, the animals were killed by rapid severing of the spinal cord. By this method we practically never found such hemorrhagic lung conditions in normal animals. In animals surviving several days these lesions were found to be in the process of absorption and they did not seem to lead to pneumonic conditions, though we should surmise that repeated lesions of this type would lower the resistance of the lung to infection. Similar lung lesions were found post-mortem in a fatally poisoned worker who may have been a victim of the acute effects of one of the guanidine derivatives.

In animals which survived several days and then died or were killed for study, there was usually seen some evidence of early toxic liver and kidney degeneration. No animals of this series survived long enough or had sufficient repeated doses to develop marked blood changes.

With most of these preparations the toxicity was rather low. There is very little danger of accidental poisoning in industry with any material having a minimum lethal dose of over 0.25 gm. per kilo, or 17.5 gm. for a man weighing 70 kilos. With limits under that point but over 0.1 gm. per kilo, there is little danger in handling the preparations if their possible toxicity is realized and reasonable precautions are taken as to dust and fume removal and personal cleanliness. It must be borne in mind, however, that personal idiosyncrasy may make exceptions to this rule, and also that fat soluble or lipoid soluble materials, if water insoluble, may poison by skin absorption in doses not toxic by mouth.

It must be emphasized that the toxicities here reported refer only to administration of solids or liquids by mouth. As previously stated, fat or lipoid soluble substances may be decidedly more toxic by skin absorption than by oral ingestion; also it must be borne in mind that the same may be true of vapor inhalation. Therefore these results do not necessarily represent the hazards of industrial exposure to vapors or to skin absorption.

TABLE 1.—SUMMARY OF TOXICITIES REPORTED

Grams per kilo of body weight for small animals (guinea-pigs and rabbits)

Guanidine and Guanidine Derivatives	
Guanidine	0.30
Diphenylguanidine	0.25
Triphenylguanidine	0.35
Diorthotolylguanidine	0.12
Diorthotolylthiourea	4.+
Aniline and Aniline Derivatives and Compounds	
Nitrobenzene by mouth	1.00
Aniline	1.75
Thiocarbanilide	4.+
Methylenedianilide	4.+
Paranitrosodimethylaniline	0.65
Paratoluidine	1.10
Condensation Products	
Formaniline	4.+
Anhydroformaldehyde-paratoluidine	2.0
Formaldehyde, Aniline, Acetaldehyde	4.+
Aniline, Acetaldehyde, Carbon bisulphide	4.+
Aniline, Paratoluidine, Butyraldehyde, Carbon bisulphide	1.7
Diphenyl and Diphenyl Derivatives	
Diphenyl	4.+
2-chlorodiphenyl	2.5
4-chlorodiphenyl	3.5
Polychlorodiphenyl, A	4.+
Polychlorodiphenyl, B	4.+
Phenylnaphthylamines	
Phenyl-alpha-naphthylamine	4.0
Phenyl-beta-naphthylamine	4.+
Tolylenediamines	
Metatolylenediamine	3.0
Paratolylenediamine	3.6

BIBLIOGRAPHY

1. Hamilton, A.: Industrial Poisons in the United States. New York, The Macmillan Co., 1925.

The chapter on "Benzene Derivatives" has been drawn on freely in compiling the first part of this paper.

2. MEYER, S.: Changes in the Blood as Reflecting Industrial Damage. THIS JOUR., 1928, *10*, 29.
3. Final Report of the Committee on Benzol. Nat. Safety Council, May, 1926.
4. SMYTH, H. F., AND SMYTH, H. F., JR.: Inhalation Experiments with Certain Lacquer Solvents. THIS JOUR., 1928, *10*, 261.
5. FLORET: Gewerbliche Vergiftungen durch Kohlenwasserstoffe der aromatischen und aliphatischen Reihe (gechlorte Kohlenwasserstoffe) und ihre Verhütung. Zentralbl. f. Gewerbehyg., 1927, N.S. *4*, 257.
6. KOBERT, R.: Kompendium der praktischen Toxikologie. Stuttgart, Ferdinand Enke, 1912, pp. 44–45.
KOBERT, R.: Lehrbuch der Intoxikationen. Second edition. Stuttgart, Ferdinand Enke, 1906, vol. 2, p. 789.
7. ISZARD, M. S.: Determination of Aniline Vapors in the Air. THIS JOUR., 1920–1921, *2*, 259.
8. HOLLEMAN, A. F.: A Text-Book of Organic Chemistry. New York, John Wiley & Sons, 1920.
9. STADELMANN, E.: Das Toluylendiamin und seine Wirkung auf den Thierkörper. Arch. f. exper. Path. u. Pharmakol., 1881, *14*, 231.
STADELMANN, E.: Die Wirkung des Toluylendiamin auf den Thierkörper. *Ibid.*, p. 422.
STADELMANN, E.: Weitere Beiträge zur Lehre vom Icterus. *Ibid.*, 1883, *16*, 118.

J. I. H.
March, 1931

STUDIES ON EXPERIMENTAL PNEUMONOKONIOSIS. VI. INHALATION OF ASBESTOS DUST: ITS EFFECT UPON PRIMARY TUBERCULOUS INFECTION

(Concluded)

LEROY U. GARDNER AND DONALD E. CUMMINGS

From the Saranac Laboratory for the Study of Tuberculosis
The Edward L. Trudeau Foundation, Saranac Lake, New York

PRIMARY TUBERCULOUS INFECTION IN PULMONARY ASBESTOSIS

To determine whether inhaled asbestos dust affects the course of tuberculous infection, the same procedures have been employed which were used in the previously reported experiments on other dusts, *i.e.*, granite (2), marble (3), carborundum (4), and quartz (5). For this purpose animals exposed to asbestos dust have been infected by causing them to inhale small numbers of tubercle bacilli of the attenuated strain R_1. By this means, in undusted guinea-pigs a self-limited infection of the respiratory tract is produced with lesions comparable with those of the "primary complex" in man. They consist of a variable number of small discrete subpleural tubercles in the lung, together with a more extensive involvement of the tracheobronchial lymph nodes. The pulmonary tubercles caseate and then the bacilli die. The caseous matter is absorbed and after a period of from eighteen months to two years, complete resolution of the entire lesion takes place (8). Macroscopic disease of the spleen and liver is almost never seen. The course of the infection in such guinea-pigs is generally nonprogressive (Figs. 18, 19, and 20).

During the period from 1920 to 1928, there were three instances of generalized tuberculosis among 251 guinea-pigs infected with this organism as controls to different dust experiments. In one of these three there was some question as to the identity of the animal. In the last three years, however, eleven instances of disseminated disease have been discovered among 148 controls similarly infected. The occurrence has been sporadic, and only one animal in a group of twenty-five or fifty such controls exhibited evidence of spreading disease. The cause of this apparent accentuation in virulence has not been ascertained.

It is hard to believe that after maintaining a constantly low degree of virulence for a period of thirty years the R_1 strain has rather suddenly been altered. As far as is known, the glycerin broth on which it has been cultivated has been prepared in the same manner as heretofore. The dosage used for inhalation infection has been maintained at a constant level. The only discoverable factor which has not

been controlled is the guinea-pigs used for the experiments. They have regularly been purchased from one farmer. On their receipt they are quarantined for a period of three weeks to avoid the introduction of epizootic pneumonia into the animal house. It had not been considered necessary to perform routine intracutaneous tuberculin tests, as no case of accidental tuberculosis had ever been recognized among this stock. It is possible that these recent cases of apparent spread of the

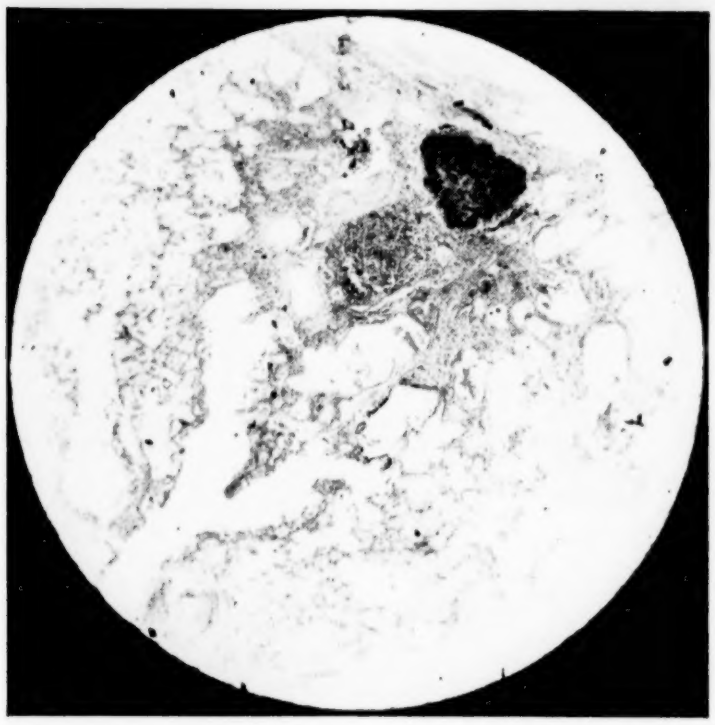

FIG. 14.—Primary subpleural tubercle partially healed by fibrosis. Below it and to the left is a nodule of hyperplastic lymphoid tissue. Slightly removed are foci of dust reaction. No spread because no contact between two lesions is established. Exposed to both dust and infection 357 days. Approximately ×20.

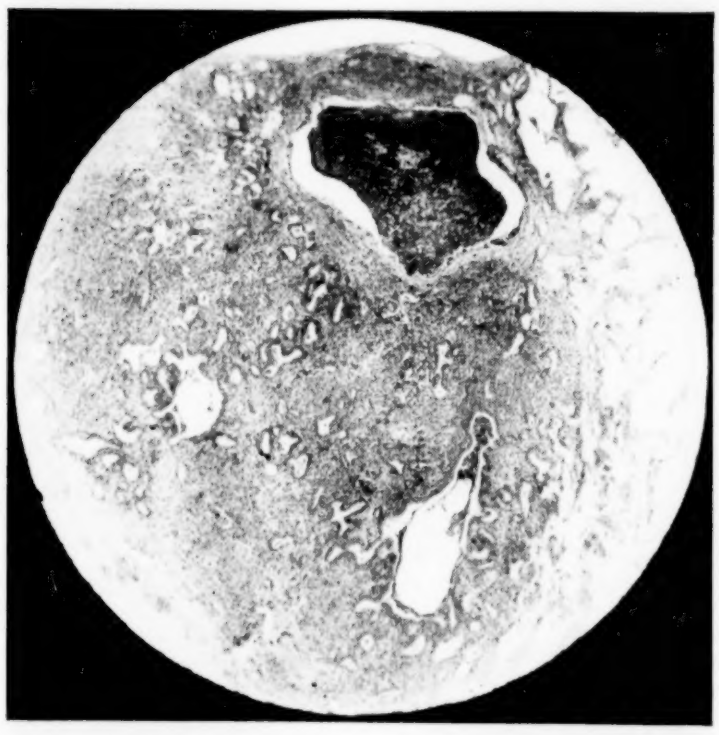

FIG. 15.—Another primary subpleural tubercle from the same lung as Figure 14, which has spread locally into an area of dust reaction. Approximately ×20.

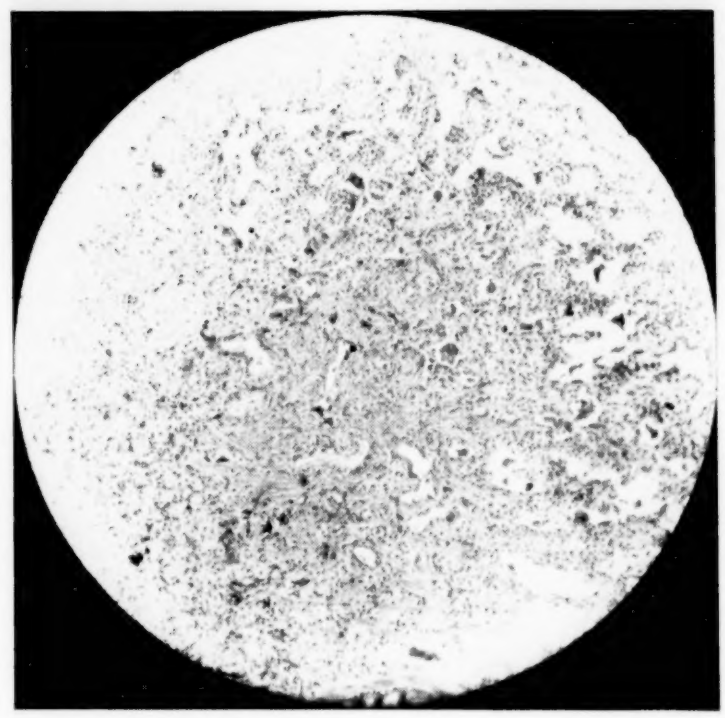

FIG. 16.—Healed fibrous tuberculosis in an area of asbestosis. Simultaneous exposure to dust and infection 838 days. Approximately ×45.

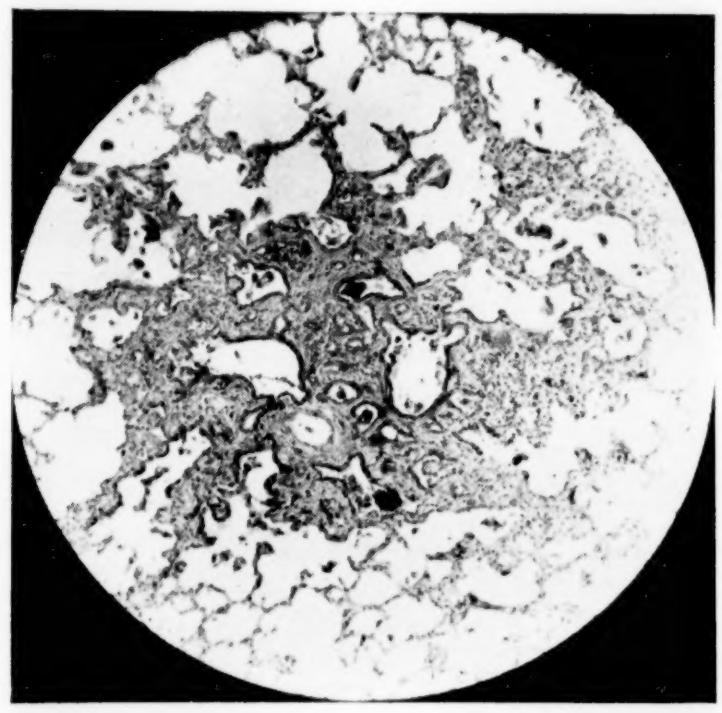

FIG. 17.—Section of uncomplicated asbestosis from same lung as Figure 16, showing more fibrosis than usual. Connective tissue stain. Approximately ×90.

low virulent artificial tuberculous infection may have been due to previous accidental infection with a more virulent strain. Generalized tuberculosis, however, has always made its appearance only after many months of residence in the animal house. Subinoculation tests are now in progress in an attempt to determine the type of tubercle bacillus responsible for this disturbing manifestation.

But in spite of these occasional instances of disseminated tuberculosis in

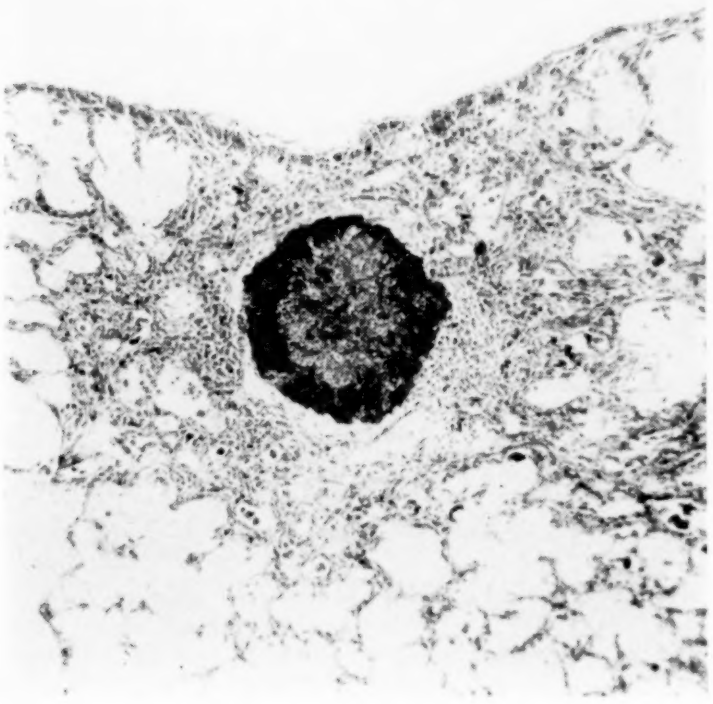

FIG. 18.—Inactive subpleural tubercle in undusted control animal; retrogression has commenced; 654 days after infection. Approximately ×110.

FIG. 20.—Healed subpleural tubercle 654 days after infection. Approximately ×110.

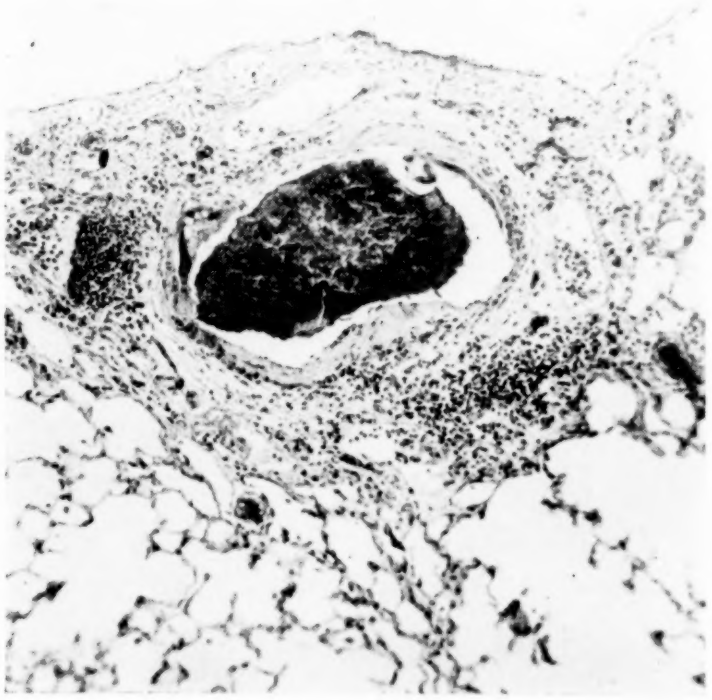

FIG. 19.—Inactive subpleural tubercle in undusted control animal; retrogression has commenced; 654 days after infection. Approximately ×110.

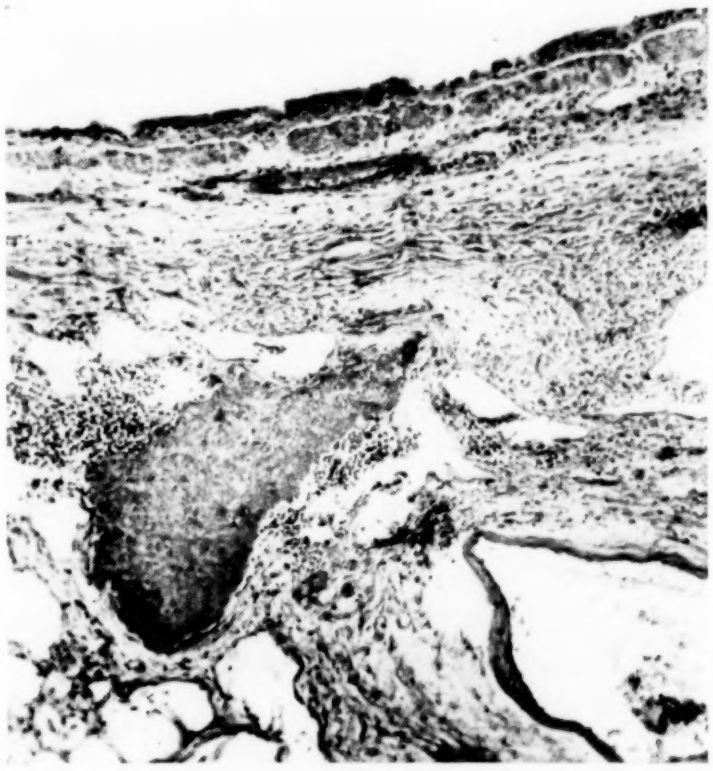

FIG. 21.—Partially healed tubercle in wall of large bronchus. Location atypical; 357 days. Approximately ×130.

the control animals, this method of testing the effect of inhaled dusts upon tuberculous infection is of value. The great majority of the controls not exposed to dust inhalation exhibit nonprogressive pulmonary tubercles which ultimately heal by resolution. On the other hand, almost every animal similarly infected and exposed to the inhalation of quartz dust dies of generalized tuberculosis, unless premature death from epizootic pneumonia, or other causes, supervenes.

In the asbestos dust experiment, two groups of guinea-pigs were infected with the attenuated R_1 strain of tubercle bacilli.[2] One lot of sixty-three normal animals were subjected to inhalation infection, and four days later forty of them were placed in the dusting room where they have been kept under the conditions already described, until death occurred. The remaining twenty-three of this group were set aside in a normal atmosphere as controls to the infection. From this experiment it was hoped that the effect of inhaled asbestos dust upon a tuberculous infection might be determined. In the animals thus exposed, asbestos particles would continue to enter the lungs during the period of tubercle formation. If solution or other chemical reaction should occur, these changes might conceivably affect the activities of the tubercle bacilli.

[2] The method of infection has been described elsewhere (2). The dosage employed for the present infection consisted of six puffs from a DeVilbiss vaporizer containing a suspension of R_1 tubercle bacilli. The water clear suspension was prepared by centrifugation and filtration through paper so that there were from ten to twenty isolated bacilli in each oil immersion field. No clumps were present. The bacilli were grown on glycerin broth, the first group for eight days, the second for twenty-one days.

A second group of twelve guinea-pigs, which had been exposed to asbestos dust for a period of two years under the conditions described above, were infected by the inhalation of an approximately equivalent dose of R_1 tubercle bacilli. At the same time twelve normal animals were infected as controls. Both the previously dusted animals and the controls were then set aside in the general animal room to be allowed to live as long as they would. This second test was designed to demonstrate whether the alterations in pulmonary anatomy and physiology produced by the inhaled dust would affect a tuberculous infection.

Infection Coincident with Dust Inhalation

Of the forty guinea-pigs originally infected and placed in the dusting chamber, thirty-one are now dead. Seven were killed, and the remainder have died of various causes: four of generalized tuberculosis, seventeen with more or less extensive epizootic pneumonia, and the rest from various accidental causes. Nine are still alive and apparently well at the time of this report, two years and three months after infection. Some evidence of spread of the tuberculous process has been observed in ten animals; in six it has been confined to the lungs, while in the other four it involved the abdominal viscera as well. Pulmonary cavity developed in four animals. In most of the cases an extension of the tuberculous infection had occurred at some time previous to death, and subsequent healing resulted in fibrosis of all the secondary lesions. In all the tuberculous animals, even those

dying with generalized disease, the lesions tend to be fibrous rather than exudative in type.

Of the twenty-three infection controls, twenty are dead. One died after 595 days, of generalized chronic tuberculosis with extensive disease in the spleen, liver, and abdominal lymph nodes. The remaining nineteen succumbed for other reasons. Twelve died of pneumonia and the others from various accidental causes. By the 244th day, the tuberculous lesions in the lungs had healed to such an extent that all evidence of caseation had disappeared. With the one exception noted, the other animals autopsied after this time showed only minute foci of scar tissue at the site of the former tubercles. In two cases there were healed fibrous tubercles of microscopic dimension in the spleen.

The distribution of the primary tuberculous foci in the lungs of the asbestosis group is somewhat atypical; not only are the usual subpleural tubercles formed, but in addition not a few foci are found in the depths of the lung, apparently originating in lymphoid tissues at bifurcation of the bronchial tree (Fig. 21). These pulmonary lesions are associated with the customary lymphatic metastasis to the tracheobronchial lymph nodes, and the development of extensive disease in that location. Many of the pulmonary tubercles are independent of foci of dust reaction and apparently when this occurs, the normal process of resolution is free to proceed. Such foci resolve completely, and ultimately disappear. Others are contiguous to or extend into areas of dust accumulated about respiratory bronchioles. When such contact is established, a reaction much more marked than that produced by either irritant alone is the result. Extensive chronic inflammation and granulation tissue develop, but the dust cells do not migrate into the interior of the tubercle, as in the case of inhaled quartz. Nevertheless, the subsequent course of the tuberculous process is altered. In some instances there is merely an interference with the usual process of resolution, so that excessive fibrosis and calcification of the necrotic central areas result. In other cases, some product is produced (possibly soluble silica) which causes renewed multiplication of the bacilli and a spread of the tuberculous process.

The extension takes place locally about the primary foci of infection (Fig. 15), and there is also metastasis of tubercle bacilli to the peribronchiolar foci of dust reaction (Fig. 7). New tubercles develop in the latter location, which are temporarily progressive and which may even break down to form small cavities. Usually, however, the process tends to come to a standstill, and ultimate healing with fibrosis and considerable anatomic deformity is the result (Fig. 16). The spreading disease is usually confined to limited areas in the lung, and death has with but one exception occurred from other causes. Such endogenous reinfections have first been encountered 203 days after the beginning of the experiment. The last animal of this group studied died on the 838th day, of endemic pneumonia which was strictly localized to one cephalic lobe. The lungs show no trace of subpleural primary foci of infection, but in the deeper portions along the course of the large bronchi are healed fibrous tuber-

cles with plaques of bone and marrow elements in their centers. These have been interpreted as the remains of primary tubercles produced by bacilli which did not reach the periphery at the time of infection because of the obstructing dust in the bronchioles. Elsewhere about respiratory bronchioles are very massive circumscribed foci of fibrosis containing the compressed remains of gland-like air spaces. Whether all of them were once the site of specific tubercle cannot be definitely determined. However, the occasional occurrence of concentric masses of scar tissue enclosing a few giant cells suggests, in some instances, the presence of tubercles. In other organs, the tracheobronchial lymph nodes and abdominal viscera, there are also many non-caseous fibrous tubercles.

Infection Superimposed upon an Established Asbestosis

Of the small group of twelve guinea-pigs infected after two years' exposure to asbestos dust, six died and three were killed during the following forty-eight days. Three of those dying presented an acute non-tuberculous pneumonia involving only one lobe of the lung, and two, an acute hemorrhagic pleural effusion.

Pulmonary tubercles were first found in the fourth animal dying on the thirty-third day; all of the remaining five showed some evidence of pulmonary infection. The majority of the tubercles occurred not in the usual subpleural zone of the lung, but in the areas where dust reaction had already been established. In some instances there are also characteristic subpleural lesions. The peribronchiolar tubercles spread in the preestablished scar tissue, and undergo extensive caseation. Early metastasis to the tracheobronchial lymph nodes occurs and the most extensive foci of reaction are found in this location. In fact, in one guinea-pig dying of an acute pleurisy on the twenty-eighth day no pulmonary tubercles can be discovered, whereas the lymph nodes are heavily infiltrated with masses of epithelioid cells. All the animals dying on and after the thirty-third day show very extensive tuberculosis of the spleen and hepatic lymph.

The infectious process exerts a very noticeable effect upon the reaction to the dust. In all but one of the animals the amount of fibrous tissue about the respiratory bronchioles is much more marked than in non-tuberculous animals exposed for the same period. In two of them, which are uncomplicated by the presence of epizootic pneumonia, the alveolar septums in wide areas peripheral to the foci of localized dust are extremely thick and fibrous.

It is of interest to note the effect of the infectious process upon the asbestosis body. The development of caseation in an area of reaction containing these structures ultimately destroys them. They lose their characteristic golden-yellow color, fail to give a Prussian blue reaction, and are apparently so completely disintegrated that not even a supporting fiber remains. Polarized light fails to reveal refractile elements. In the walls of small tuberculous cavities developing in foci of dust reaction, the various steps in the destruction of the asbestosis body can be studied. The cause of their disappearance has not been determined but it is possible that the change in

hydrogen ion concentration incident to caseation may favor solution of the foreign bodies.

Discussion

The physical and chemical properties of asbestos are so unlike those of any other dust previously studied that, when inhaled, particles of this substance provoke an unusual type of reaction in the lung.

Localization of Dust

The fact that a fiber as long as 100 and even 200 microns can be inhaled and ultimately reach the finer branches of the bronchial tree is surprising and controverts the accepted ideas of the effectiveness of the upper respiratory protective mechanism. Whether such structures enter the lung in full extension, or whether they are partially coiled has not yet been determined. Coiled fibers do not occur in the dust itself. In any case, it is difficult to imagine a structure of such dimension making its way against the normal current of ciliary action. It is generally assumed that prolonged inhalation of dust provokes a chronic bronchitis, but in the case of asbestos dust no morphologic evidence of such a change has been observed within a period of two and one-half years. It is quite possible that the physiologic activities of the ciliated epithelium may be altered without demonstrable anatomic changes. In support of such a view is the fact that very long fibers have not been discovered in the deeper portions of the lung until the dust inhalation has been in progress for several months.

Unlike other dusts, asbestos fibers do not, at least for two years, penetrate into the ultimate divisions of the primary units of the lung; the major part of them come to rest in the respiratory bronchioles. It will be recalled that these structures are lined, not by ciliated, but by smooth cuboidal epithelium, and that at intervals along their walls are the openings of a variable number of alveoli. As a result, the bronchial tube at this point is no longer smooth walled, but is very irregular in contour. This roughness is probably at least one of the factors responsible for the arrest of the elongated spicules and fibers which catch in the openings of the lateral alveoli. In marked contrast with this is the behavior of a particulate dust like quartz, the major portion of which passes through the lumen of a respiratory bronchiole and enters the alveolar duct and its further ramifications.

Having come to rest, the asbestos particles are ingested by free alveolar phagocytes. As so much of the material consists of elongated fibers, giant cells are very prone to develop. Within the cytoplasm of these cells the chemical changes responsible for the development of that unique structure, the asbestosis body, occur. The process requires a period of approximately two months before the characteristic alterations are produced.

Chemical Reaction and Asbestosis Body

That the changes in the inhaled material are truly chemical in nature is indicated by the appearance of dissolved iron in the cytoplasm of certain phagocytes at a time previous to the appearance of the asbestosis body. The presence of the iron imparts a diffuse yellow coloration to the cells

and it reacts specifically with potassium ferrocyanide and dilute hydrochloric acid.

As previously noted, study of the structure of the asbestos fibers indicates that iron occurs in intimate combination with silica, and even in the best grade of chrysotile it actually replaces some of the magnesium. Chemical changes involving one constituent of the asbestos molecule must affect its other components. The presence of iron uniformly distributed throughout the cytoplasm of a phagocyte may indicate either hydrolysis of the ingested dust or solution of at least part of the original constituents and a subsequent release of silica. This silica may exist in any of several physical states, as soluble, colloidal, or insoluble silica, or as soluble silicate. By study of the chemical structure and the method of formation of the asbestosis body, it should be possible to discover the actual mechanism by which structural alteration of an inhaled silicate occurs.

The asbestosis body has been subjected to various chemical tests by several investigators. Cooke (9) and McDonald (10) first described this unusual foreign body and stated that it did not stain with aniline dyes, but gave a marked iron reaction. They also noted that it was not doubly refractile. McDonald (10) and Simson (11) also presented a theory for the formation of the golden-yellow bodies from asbestos. Stewart (12) claimed the presence of asbestosis bodies in histologic sections of lung as diagnostic of pulmonary asbestosis. Cooke (13) later called attention to the relation between the iron content of asbestos dust and the iron reaction of asbestosis bodies. He noted that they were not digested by trypsin and that they contained less iron than chrysotile. He also noted that they failed to cast a characteristic pattern when treated by the X-ray diffraction method of Bragg, though a central fiber could be demonstrated with a dissecting microscope. He therefore concluded that they were fibers of vegetable origin covered with colloidal aggregates of adsorbed blood proteins. Stewart (14) replied to Cooke's suggestions, and stated that all the reported cases of asbestosis had exhibited these curious structures. Stewart (15) later noted that antiformin did not destroy them. Gloyne (16) stated that the asbestosis body withstood calcination, but lost its golden-yellow sheath after treatment with concentrated sulphuric acid, leaving a thin central fiber. He reported the body as refractile on dark ground illumination.

We have been able to confirm many of these findings, and we have also attempted to prove conclusively whether or not the asbestosis body contains organic matter. Since it resists calcination without change in configuration or color, and since it is not digested with either pepsin and hydrochloric acid or trypsin, it is not to be regarded as protein in nature. Moreover, asbestos fibers fail to adsorb proteins when placed in blood or peptone broth media for several months. This curious structure is not soluble in any of the usual organic solvents[3] and fails to show charring with sulphuric

[3] It will be recalled that the asbestosis bodies and even their supporting fibers ultimately disappear in an area of tuberculous necrosis. The explanation is not entirely clear but solution may be due to a high degree of alkalinity created in the degenerated cells.

J. I. H.
March, 1931

acid. Consequently, it is considered to be an inorganic structure. Inasmuch as a central fiber can be readily demonstrated in nearly all instances, the body is undoubtedly derived from asbestos.

The marked iron reaction of the asbestosis body is not to be confused with that obtained on the dust previous to contact with the animal body. In its original state the iron in the fibers is almost wholly in the ferrous state, while in the asbestosis body only ferric iron can be detected. This structure is therefore regarded as an oxidation product of the original dust.

In an attempt to prove whether the iron of the asbestosis body was already present in the fiber or whether it was derived from animal tissues, the experiment was made in which asbestos fibers, freed from iron by leaching with hydrochloric acid, were injected into the subcutaneous tissues of guinea-pigs as noted above. It was unsuccessful because the acid treatment rendered the asbestos soluble in the body fluids, and no significant reaction occurred. One month after the injection, practically no trace of the fibers could be discovered and only a very slight fibrosis marked the site.

Treatment of asbestos fibers for the determination of free silica disclosed the ease with which the chrysotile molecule could be opened. If enzymatic action or hydrolysis of the fiber is assumed to take place in the presence of body fluids, it is quite possible that the ferrous or ferric silicate existing in the fiber is oxidized and hydrolyzed directly to produce the asbestosis body.

As evidence that hydrolysis or partial solution of the inhaled dust is responsible for the development of the asbestosis body, the following observations may be cited. When studied by Bragg's X-ray technic, asbestos fibers exhibit a characteristic crystalline pattern which is lost upon treatment with acid. Cooke (13), using the same method, showed that asbestosis bodies exhibit no crystalline structure. Ray and Ganguly (17) state that precipitates formed from solutions of ferric chloride and sodium silicates, which are identical in color with the asbestosis body, also yield no crystalline pattern by X-ray. As further evidence of physical alteration is the fact that the asbestos fiber is highly refractile in polarized light, whereas the asbestosis body has completely lost this property.[4]

We have attempted to reproduce the

[4] It has been assumed that the demonstration of iron in the outer coating of the asbestosis body is evidence of hydrolysis of the original asbestos fiber on which the body formed. But actually this finding does not constitute definite proof unless an endogenous source from blood pigment can be excluded. We have thus far failed to eliminate this source of iron. However, the demonstration of magnesium in the asbestosis body would furnish equally convincing proof of hydrolysis and the objection that it was derived from the tissues could hardly be raised, for the amount of this substance in the body is extremely small. An attempt has, therefore, been made to develop a microchemical test for magnesium. It has been found that both asbestos fibers and asbestosis bodies are definitely stained a light blue-green color when treated on microslides with a dilute hydrochloric acid solution of para-nitro-benzene-azo-resorcinol followed by a bath of sodium hydroxide.

The presence of magnesium in the outer layers of the asbestosis body is convincing proof of hydrolysis of the inhaled dust. This color reaction is given only by magnesium, cobalt, and nickel, substances which do not occur in sufficient concentration in the tissues to cause reaction. Therefore, endogenous sources for the coating material of the asbestosis body are assumed to have been eliminated.

asbestosis body from asbestos fibers by immersing them for prolonged periods in buffer solutions of various hydrogen ion concentrations, both above and below neutrality. The solutions have been adjusted with agar and silica gel to a viscosity near that of the cell. Oxidizing agents have been added and the reaction has been allowed to proceed in the incubator for six months. No golden-yellow structures were obtained.

We have, however, been able to reproduce the structure of the asbestosis body quite closely by other means. Iron free asbestos fibers are impregnated with an iron salt by heating to dryness in a dilute ferric chloride solution and ignited. The fibers are then placed in a solution of sodium silicate. The iron salt adhering to the fibers reacts rapidly with the silicate, producing a curiously shaped golden-yellow structure which very closely resembles the asbestosis body (Fig. 13, Nos. 1 and 2).

A similar reaction is involved in the production of the familiar phenomenon, the "silicate garden" (18). If a crystal of ferric chloride is added to a solution of sodium silicate, golden-yellow filaments, often several inches in length, rapidly develop, which exhibit irregular swellings and buds. Ferric silicate is probably formed. This product is partially hydrolyzed, producing a thin wrinkled skin of silicic acid streaked with ferric hydroxide. This skin, acting as a semipermeable membrane, permits diffusion to take place and a high osmotic pressure develops with a consequent rupture of the membrane. Further hydrolysis occurs and the reaction repeats itself until finally the membrane becomes impermeable.

Microscopic structures of fibrous character, suggesting asbestosis bodies, have been produced both with dilute solutions of an iron salt and a soluble silicate, and with iron and silica sols (19).

These observations lead us to conclude that the asbestosis body is a derivative of the crystalline asbestos fiber which has been oxidized and hydrolyzed to a golden-yellow amorphous structure. As such, it can be regarded as the first direct evidence of altered chemical composition in an inhaled silicate dust.

In the animal body, the evidence thus far accumulated would indicate that the lung of the guinea-pig and of man furnishes the most favorable environment for the development of the asbestosis body. Asbestos fibers transported from the lung to the tracheobronchial lymph nodes do not ordinarily appear to produce these structures. Possibly prolonged residence in the lung has deprived them of their capacity for further reaction, or possibly some necessary factor is lacking in the lymph nodes. It is believed that the few asbestosis bodies found in the nodes were transported after attaining full development in the pulmonary air spaces. Injection of asbestos dust into the peritoneal cavity of guinea-pigs has failed to produce characteristic structures in any quantity. In the subcutaneous tissues they do develop, but the period of incubation is nearly twice as long as that required in the lung. Practically no asbestosis bodies have formed in the lungs of rabbits and albino rats. The necessary environmental factors which are lacking in

any of these situations are entirely unknown.

Attempts to determine whether the asbestosis body will develop in the animal when any of the theoretically necessary elements are lacking have thus far met with little success. The experiments *in vitro* indicate that crystalline ferric or ferrous silicate in a medium of proper viscosity and a fiber for support are probably essential to the production of these structures. In our attempt to determine whether iron derived from the tissues could be substituted for the iron inherent in the chrysotile molecule, we have been defeated by the fact that the manipulation necessary to remove the iron from the fiber apparently renders that structure soluble in the body fluids.

The injection and the inhalation of dark Barre granite dust, which contains both free silica and iron, have never resulted in the formation of structures in any way suggesting asbestosis bodies. The solution of iron can be demonstrated by microchemical tests and it generally is assumed that the silica is also ultimately dissolved. Intraperitoneal injections of granite from Lorain County, Ohio, have been made because this material contains iron in the unusual form of ferrous carbonate. No suggestion of an asbestosis body has been discovered. In the light of subsequent studies, it is now recognized that the peritoneum is not a proper location for such a test, and subcutaneous inoculations have already been made with this substance, but it is too early to report on them.

Thus far, it would appear that the mere presence of iron and free silica is not sufficient for the production of asbestosis bodies.

Pulmonary Fibrosis

That the long-continued inhalation of asbestos dust is responsible for the development of pulmonary fibrosis is now unquestioned. From many parts of the world come radiographic reports of fine fibrosis in the lungs of persons exposed by occupation to the inhalation of this substance. The findings from at least ten postmortem examinations have furnished reliable evidence that the shadows seen in the roentgenogram are produced by areas of fibrous tissue (20). In guinea-pig experiments reported in this paper, it has been shown that the fibrosis begins in those portions of the lung where the dust is localized, and that this reaction can be detected in X-ray pictures after about two years' exposure to the concentration of dust used (Fig. 3).

The earliest evidence of proliferation of fibroblasts has been encountered approximately 500 days after the commencement of the dust exposure. It first appears in the immediate vicinity of the largest accumulations of dust cells, which occur in the respiratory bronchiole and its adjacent alveoli. As increasing amounts of dust accumulate in the walls of these structures, the amount of fibrosis is likewise increased. In the otherwise normal guinea-pig, migration of dust cells to intrapulmonary lymphoid tissue has not been encountered, and in consequence the nodular foci of fibrosis, which are so characteristic of the reaction to quartz dust, fail to develop. The occurrence, in one of the last animals killed, of a more diffuse fibrosis peripheral to the point of

maximum dust deposition suggests that collapse induration incident to occlusion of respiratory bronchioles may play an important part in the later stages of the disease process.

Asbestos dust, a silicate of magnesium, is relatively soluble in fluids comparable with those of the tissues. The asbestosis body develops only after solution has occurred in the proper environment. The development of these structures in the lungs of guinea-pigs and of human beings is the first direct evidence of solubility of an inhaled dust known to produce pulmonary fibrosis. It still remains to be demonstrated whether a dissolved substance is the factor responsible for the multiplication of fibroblasts in pneumonokoniosis. In this connection the evidence from asbestos inhalation in the rabbit should prove most valuable. If, after a proper period of exposure, typical asbestosis bodies should still be lacking, and if fibrosis should likewise fail to develop, it would be logical to conclude that fibroblasts were not stimulated because no solution of the dust had occurred. Thus far, the reaction of the rabbit has only been studied during an exposure period of 330 days. The only sign of asbestosis body formation is an irregular swelling of certain fibers which give microchemical tests for iron; evidence of fibrosis is entirely lacking. The 500-day period of exposure, when true fibrosis appeared in the guinea-pig, is anxiously awaited.

Comparison between Reactions to Asbestos and to Other Dusts

Granite dust, which is known to provoke the formation of extensive fibrosis in human beings, appears to act more slowly than asbestos in the guinea-pig. After 910 days, the maximum period of experimental exposure to granite, the lungs show large masses of dust filled phagocytes lying in the lumen of the alveoli along the alveolar ducts and atria. In the adjacent septums there is a slight thickening due to the presence of lymphoid and monocytic cells, but fibrosis is entirely lacking. The significant feature is the *absence of dust particles in the connective tissues of the lung*. In the tracheobronchial lymph nodes, on the other hand, dust accumulates in such quantities that fibrosis develops within one year, and after two years' exposure the lymphoid tissues are largely destroyed by a series of true silicotic nodules. It has been postulated that fibrosis has not developed in the lung of the animal exposed to granite because the dust particles do not come into sufficiently intimate contact with the fibroblasts of the alveolar walls. In the lymph nodes, on the other hand, fibrosis begins early, and typical silicotic nodules are produced because heavy concentrations of dust are brought into close proximity with connective tissue elements.

The conditions of the experiments with granite and asbestos dusts would theoretically favor a greater reaction in the former case. The concentration of granite dust was over eight times as great as that of asbestos (287,700,000 particles less than 1.5 microns in diameter per cubic foot of air for granite and only 34,466,000 particles of the same size for asbestos). However, the greater solubility of magnesium silicate, together with its peculiar localization, probably is the

factor responsible for the production of the more rapid and extensive pulmonary fibrosis by asbestos dust.

Quartz, which is generally accepted as the most active of all the nontoxic dusts which are inhaled in industry, is much less easily dissolved than asbestos, and yet it will produce extensive fibrosis in the lungs of experimental animals more rapidly. In guinea-pigs, pulmonary fibrosis, and even necrosis, has been observed after one year's exposure to quartz dust; in rabbits, typical silicotic nodules are formed in eighteen months. The difference in the rate of reaction to quartz and asbestos has been ascribed to several causes. With equivalent concentrations of dust in two given atmospheres, more quartz than asbestos particles will be inhaled and reach the parenchyma of the lung. This is presumably due to the relative differences in the shape and the surface characteristics of the particles. After phagocytosis, quartz particles seem to possess the ability to stimulate a rapid migration of the dust cells, so that they concentrate large masses of dust about nodules of intrapulmonary lymphoid tissue. Here again, but by a different mechanism, the foreign bodies are collected in circumscribed areas. Subsequently, perhaps as the result of solution of the quartz, a local proliferation of fibroblasts takes place.

Carborundum dust has produced no pulmonary fibrosis after exposure as long as three or four years. In the lung, it is treated much like granite dust, collected in phagocytes which remain within the air spaces, so that the dust cells establish no contact with the underlying connective tissue. In the tracheobronchial lymph nodes, carborundum does excite fibrosis, and structures suggesting silicotic nodules have been discovered. As in the case of granite, proliferation of fibroblasts occurs at the point where they are in intimate contact with sufficient numbers of dust laden phagocytes. Whether solution of carborundum by the body fluids is possible has never been determined.

From these observations on the reaction to various types of dust, an hypothesis has been developed that fibroblasts proliferate in response to sufficient concentrations of a soluble substance liberated by the action of the phagocytes on the included dust particles. The soluble substance probably diffuses through the membrane of the intact phagocytes, but it is only stimulating to fibroblasts in the immediate vicinity. The undefined soluble substance, possibly silica in some form, is rapidly neutralized after leaving the cells in which it is produced, and it exerts no effect upon more remote connective tissue elements. The process of neutralization may be chemical in nature or it may be a result of absorption by some other type of cell which is itself unaffected.

Theoretical Consideration of Pneumonokoniosis

In a previous paper dealing largely with the reactions to granite and carborundum dusts (21), it was suggested that the primary lesion of pneumonokoniosis consists in the development of an obstructive fibrosis in the tracheobronchial lymph nodes attended by stasis in the afferent lymphatics in the lung, and ultimately followed by a perilymphatic fibrosis. When only these two types of dust had

been studied, such an hypothesis looked tempting, for the only trace of fibrosis in the lung in these cases was situated about the large lymph trunks. Masses of dust filled phagocytes lay for many months in the air spaces, and apparently provoked not the slightest reaction of the connective tissues. But subsequent experimental investigation has modified this concept; it is now believed that fibrosis develops wherever sufficient amounts of dust of the proper type come into intimate contact with fibroblasts. The primary obstructive lesion in the tracheobronchial lymph nodes occurs with quartz inhalations but not with asbestos. Undoubtedly such a lesion hastens the development of reaction in the lung, for it reduces elimination and concentrates the dust, but it is not essential to the production of pulmonary fibrosis.

One further point in connection with the state of the lymphatics deserves comment. In experimental asbestosis, both in the guinea-pig and in the rabbit, the lymph vessels are widely dilated after as short an exposure as from thirty to sixty days. Inhaled asbestos dust is not transported to the tracheobronchial lymph nodes in any considerable quantity, and no obstructive lesion has been produced at this time. Therefore in this case the dilatation of these vessels must have some other cause; possibly it is merely a physiologic response to intrapulmonary irritation. Asbestos filled phagocytes do not enter these channels because they are apparently relatively inactive, but epithelioid cells containing tubercle bacilli may leave the lung in great numbers. Apparently the lymphatic channel of escape is open to the asbestos dust cells, but for causes inherent in themselves, they do not migrate to these vessels.

Tuberculous Infection and Asbestosis

Where inhalation infection is instituted simultaneously with commencement of dust exposures, the majority of inhaled tubercle bacilli localize in small peripheral lymphoid masses in a zone immediately beneath the pleura. This is characteristic of such infection with the organism used in the experiment. A certain number come to rest atypically in the deeper lymphoid tissues along the intermediate bronchi, possibly because they are mechanically arrested by inhaled dust particles. The majority of the tubercles formed undergo spontaneous resolution which is the outcome of the infection in most normal control animals. A certain number of tubercles by accident localize in proximity to foci of localized dust. If the contact is sufficiently intimate, these tubercles spread locally and more remotely into foci of dust reaction about respiratory bronchioles. A new crop of tubercles develop in this location, and these may progress and even form small cavities, but the ultimate outcome is usually healing with more or less extensive fibrosis and calcification. The tuberculous process is practically never generalized throughout the lung, but is generally confined to small nodular foci. Blood stream metastasis is usual and macroscopic disease in the spleen and sometimes in the liver is common, an occurrence almost never found in infection controls. In the thirty-one animals of this group dead at the time of this report, a spread of the tuberculous infection occurred in 32.2 per cent. In

40 per cent. of them, the tuberculosis had subsequently more or less completely healed by fibrosis. It seems probable that in the remaining members of the group a similar result would have been attained had they not died prematurely from accidental causes.

Where inhalation infection was superimposed upon a well-established asbestosis of approximately two years' standing, the primary localization of the tubercles was atypical. Many of the tubercle bacilli were mechanically held up by the reaction to the dust in the respiratory bronchioles. It would appear that many, perhaps the majority, of these organisms were phagocytosed and carried directly into the distended lymphatic trunks. The earliest manifestation of tubercle formation occurred not in the lung, but in the tracheobronchial lymph nodes, a condition which has never before been observed in experimental inhalation infection. Some organisms localized in the foci of dust reaction and then produced tubercles; a certain number of bacilli passed to the periphery, where typical subpleural lesions developed. The major portion of the guinea-pigs comprising this group died or were killed during a period of forty-eight days after infection. Within this time not much extension of the intrapulmonary infection had occurred. The tracheobronchial lymph nodes were heavily involved in all animals dead after the thirty-third day. It is too early to state what will be the ultimate outcome of infection superimposed upon a preestablished asbestosis.

From these experiments, it would appear that inhaled asbestos dust is capable of exerting some stimulating effect upon tubercle bacilli of attenuated virulence. To exert this effect, the dust and the tubercle bacilli must be brought into rather intimate association. This occasionally occurred when the infection and the onset of the dust inhalation were simultaneous; it was much more common where infection was superimposed upon an already established asbestosis. The dust, perhaps by the action of its dissolved products or by alteration in tissue reaction, initiates a renewed proliferation of the tubercle bacilli, and the infection spreads. But this stimulating effect is only temporary and ultimately the foci of new disease tend to heal by fibrosis. In this respect, inhaled asbestos dust differs from quartz, which incites continued and progressive multiplication of the bacteria and consequent spread of disease.

As has been observed with the other types of dust (granite and carborundum), the presence of asbestos in the lung of an animal infected with tubercle bacilli promotes more extensive fibrosis than is produced when either the organism or the dust is acting alone. Where the two irritants are concentrated in the same area, each provokes the formation of granulation tissue which ultimately organizes to form fibrosis. But even in areas of dust reaction remote from the site of tubercle, the fibrosis is excessive, an effect for which explanation is lacking. (Figs. 15 and 16.)

Summary

Guinea-pigs have been exposed for eight hours daily for periods as long as two and one-third years to an atmosphere containing approximately thirty-five million particles per cubic foot of

asbestos dust (Canadian chrysotile) 1.5 microns and less in diameter. Rabbits and albino rats have likewise been exposed for shorter periods (330 days).

The experiments demonstrate that fibrous structures at least as long as 200 microns can pass the protective mechanism of the upper respiratory tract and enter the lung. Anatomic evidence of injury to this mechanism is wanting.

Inhaled asbestos dust does not penetrate to the terminal alveoli of the lung as is the case with a particulate substance such as quartz. The major portion is held up in the respiratory bronchioles. There phagocytosis takes place, and there the material remains localized, at least for a period of two and one-third years. Phagocytes containing asbestos particles migrate into the lateral alveoli given off from the walls of the bronchioles, and considerable numbers ultimately penetrate the adjacent connective tissues. In the guinea-pig, transportation of dust particles to intrapulmonary and mediastinal lymphoid tissues is so slow that changes in these structures play little part in the early development of asbestosis. In the rabbit, dust cells begin to appear in the lymphoid tissues of the lung within sixty days after the commencement of the inhalation; thereafter, they continue to migrate in increasing numbers.

In the guinea-pig, fibrosis in the walls of the respiratory bronchioles and their lateral alveoli is first manifested after 500 days' exposure. Thereafter this type of reaction progressively increases in intensity and extent. The resulting atelectasis is responsible for a gland-like appearance, which is due to the contraction of the included alveoli and a consequent compression of the epithelial lining cells. In the rabbit sufficient time has not yet elapsed for fibrosis to be expected.

In the lungs of guinea-pigs, asbestosis bodies, apparently identical with those described in the human being, have developed after an exposure of approximately seventy days. In the rabbit these structures have not been discovered after exposures as long as 330 days. In the albino rat they are very rare. Only two small typical forms have been discovered in one animal exposed for seventy days. The prevalence of chronic infections of the lung in all members of this series is possibly responsible.

Asbestosis bodies apparently fail to form in the tracheobronchial lymph nodes of guinea-pigs. They may be transported in small numbers to these tissues and to areas of chronic pleurisy. They have not been discovered in the peritoneal cavity 100 days after injection of dust. In the subcutaneous tissue of the groin, typical forms were found 102 days after the injection of 3 mg. of dust.

Asbestosis bodies are not present in asbestos dust previous to contact with animal tissues. They are produced by oxidation and hydrolysis of the chrysotile molecule. The formation of these structures is the first direct evidence that the body is capable of effecting changes in inhaled silicate particles. The chemical processes involved have been discussed *in extenso*. Attempts at artificial production of asbestosis bodies *in vitro* have been partially successful; solutions of ferric chloride and sodium silicate have been made to combine in the presence of a fiber to pro-

duce more or less typical forms. The asbestosis body is therefore analogous to the well-known "silicate garden." Attempts to produce asbestosis bodies *in vivo* by the injection of dusts containing iron salts and silicates have thus far failed. The failure to produce them in tissues other than the lung and subcutaneous tissues of guinea-pigs and man has not been explained.

Comparison between the localization and the reaction to asbestos and other types of inhaled dusts has been shown. The points at which inhaled dust is localized in the lung or lymph nodes vary with the type of dust. Granite remains within the pulmonary air spaces and produces no local reaction of fibroblasts for several years. In the tracheobronchial lymph nodes characteristic silicotic nodules develop within two years. Carborundum likewise has failed to affect the stroma of the lung even in four years, but fibrosis in the lymph nodes has been observed. Quartz is rapidly concentrated by migrating phagocytes in the pulmonary and mediastinal lymphoid tissues. In these places it provokes an early and rapid multiplication of fibroblasts. Asbestos, as it is inhaled, is concentrated in respiratory bronchioles and their lateral alveoli. Phagocytes carry it into the walls, where fibroblasts are stimulated.

Lymph stasis plays little part in early asbestosis; the structure of this dust tends to localize it within the lung from the start. Lymph vessels are dilated in the absence of detectable obstruction. The dilatation may be a direct result of pulmonary irritation.

Primary tuberculous infection is influenced only to a limited degree by inhaled asbestos. This conclusion has been reached after inhalation infection with attenuated tubercle bacilli (strain R_1). In normal guinea-pigs such infection produces tubercles in the lung and tracheobronchial lymph nodes comparable to the "primary complex" in man. The lesions caseate and the pulmonary foci heal by resolution. Spread of the infection with macroscopic disease in other viscera is very rare. In the asbestos experiment, one group of guinea-pigs was infected at the outset of dust inhalation; a second group, two years after the commencement of dust exposure. In the first series, 32.2 per cent. of the animals showed some evidence of spreading tuberculosis. New disease began as a local extension from primary tubercles and metastasis to areas where dust reaction had occurred was common. Rarely small cavities developed in the secondary foci. The tendency to healing by fibrosis was marked; at autopsy 40 per cent. of the cases showed healed fibrous tuberculosis; macroscopic disease in the spleen and sometimes in the liver was common. The contrast with animals similarly infected but exposed to quartz dust is marked. In them every exposure longer than five months resulted in generalized chronic tuberculosis of the lungs and other viscera.

For the second asbestosis group infected, the localization of tubercles was atypical. Many bacilli were trapped in foci of dust reaction. Some produced local tubercles; others immediately entered the dilated lymph vessels and were transported to the tracheobronchial lymph nodes. Tuberculosis in these nodes sometimes occurred without involvement of the lung. Early disease in the spleen and hepatic lymph nodes was the rule.

The ultimate outcome of infection in this group has not yet been observed.

The combined action of asbestos dust and tubercle bacilli in the lung produced more fibrosis than did either agent acting independently.

BIBLIOGRAPHY

1. Anderson, H. V., and Clark, G. L.: Application of X-Rays in the Classification of Fibrous Silicate Minerals Commonly Termed Asbestos. Indust. and Engin. Chem., 1929, *21*, 924.
2. Gardner, L. U.: Studies on the Relation of Mineral Dusts to Tuberculosis. I. The Relatively Early Lesions in Experimental Pneumokoniosis Produced by Granite Inhalation and their Influence on Pulmonary Tuberculosis. Am. Rev. Tuberc., 1920–1921, *4*, 734.
3. Gardner, L. U., and Dworski, M.: *Idem.* II. The Relatively Early Lesions Produced by the Inhalation of Marble Dust and their Influence on Pulmonary Tuberculosis. *Ibid.*, 1922–1923, *6*, 782.
4. Gardner, L. U.: *Idem.* III. The Relatively Early Lesions in Experimental Pneumokoniosis Produced by Carborundum Inhalation and their Influence on Pulmonary Tuberculosis. *Ibid.*, 1923, *7*, 344.
5. Gardner, L. U.: Studies on Experimental Pneumonokoniosis. V. The Reactivation of Healing Primary Tubercles in the Lung by the Inhalation of Quartz, Granite and Carborundum Dusts. *Ibid.*, 1929, *20*, 833.
6. Stewart, M. J., and Haddow, A. C.: Demonstration of the Peculiar Bodies of Pulmonary Asbestosis ("Asbestosis Bodies") in Material Obtained by Lung Puncture and in the Sputum. Jour. Path. and Bacteriol., 1929, *32*, 172.
7. Cummings, D. E.: Studies on Experimental Pneumonokoniosis. IV. The Separation of Particulate Matter Smaller than Screen Sizes into Graded Fractions. This Jour., 1929, *11*, 245.
8. Gardner, L. U.: Healing by Resolution in Experimental Pulmonary Tuberculosis. Am. Rev. Tuberc., 1922–1923, *6*, 163.
9. Cooke, W. E.: Pulmonary Asbestosis. Brit. Med. Jour., 1927, *2*, 1024.
10. McDonald, S.: Histology of Pulmonary Asbestosis. *Ibid.*, p. 1025.
11. Simson, F. W.: Pulmonary Asbestosis in South Africa. *Ibid.*, 1928, *1*, 885.
12. Stewart, M. J.: The Immediate Diagnosis of Pulmonary Asbestosis at Necropsy. *Ibid.*, 1928, *2*, 509.
13. Cooke, W. E.: Pulmonary Asbestosis. *Ibid.*, p. 585.
14. Stewart, M. J.: Pulmonary Asbestosis. *Ibid.*, p. 675.
15. Stewart, M. J.: A Method of Examining the Sputum for Asbestosis Bodies. *Ibid.*, 1929, *2*, 581.
16. Gloyne, S. R.: The Presence of the Asbestos Fibre in the Lesions of Asbestos Workers. Tubercle, 1929, *10*, 404.
17. Ray, R. C., and Ganguly, P. B.: The Optimum Conditions for the Formation of Silica Gel from Alkali Silicate Solutions. Part I. Jour. Phys. Chem., 1930, *34*, 352.
18. Vail, J. G.: Soluble Silicates in Industry. New York, The Chemical Catalog Co., Inc., 1928, p. 79.
19. Moore, B., and Evans, W. G.: On Forms of Growth Resembling Living Organisms and their Products Slowly Deposited from Metastable Solutions of Inorganic Colloids. Proc. Roy. Soc. London, Series B., 1915–1917, *89*, 17.
20. Merewether, E. R. A., and Price, C. W.: Report on Effects of Asbestos Dust on the Lungs and Dust Suppression in the Asbestos Industry. Part I. London, H. M. Stationery Office, 1930.
21. Gardner, L. U.: Tuberculous Infection and Tuberculosis as Modified by Experimental Pneumonokoniosis. Tubercle, 1924–1925, *6*, 336, 443.

J. I. H.
March, 1931

THE SO-CALLED CYANIDE RASH

The Editor,
Journal of Industrial Hygiene.

Sir:

Braddock and Tingle's article on "So-Called Cyanide Rash in Gold Mine Mill Workers," in the JOURNAL for September, 1930, interests me because I can add sixteen years' personal experience and observations from New Zealand and the state of Western Australia to the other countries listed. As a cyanide salt we first used potassium cyanide, but this was superseded, as in all countries, by sodium cyanide. The solutions were a stock liquor of up to 20 per cent., from which the working solutions of 0.1 to 0.2 per cent. were made. Ordinarily, these working solutions were cold, but when in some plants the roasted ore was mixed therewith, the solutions became hot and remained warm to the end of the process. An odor of cyanide pervades every gold or silver ore treatment plant, and renders smoking unpleasant. As Braddock and Tingle conclude, the rash appears to be a dermatitis—that is, it is a superficial ailment; the blood is not a factor. I had the rash more or less severely on the arms and face, especially at the hair roots; others had it there and on the legs. Drinkers and nondrinkers, and smokers and nonsmokers alike were affected, but not everyone employed around cyanide. As far as my observation went, as one handling cyanide salts and solutions, also as a foreman and metallurgist, there was no lack of personal cleanliness among the men, as suggested. Some men became immune to the rash after being affected for a time; others had to change their jobs. The wearing of rubber gloves helps, but they induce sweating which often starts a rash. As a rule, we used potassium permanganate solution, but zinc oxide ointment also alleviated the trouble which, as Braddock and Tingle conclude, is probably due to irritation by the strong caustic, excited by lime used in the process. We never considered it as cyanide poisoning, but simply a skin outbreak.

M. W. VON BERNEWITZ

Pittsburgh, Pa.
December 19, 1930.

BOOK REVIEW

QUANTITATIVE ORGANIC MICROANALYSIS. By *Fritz Pregl*, D.Sc., Ph.D., Professor of Medicinal Chemistry; Principal of the Medico-Chemical Institute of the University of Graz; Corresponding Member of the Academy of Sciences, Vienna. Second English edition. Translated from the third revised and enlarged German edition by Ernest Fyleman, B.Sc., Ph.D., F.I.C., Consulting Chemist, Sutton, Surrey. Cloth. Pp. 237 with illustrations and index. Philadelphia: P. Blakiston's Son & Co., Inc., 1930.

Within recent years the noticeable trend in analytic chemistry has been in the direction of microanalysis. It has become increasingly important to know more and more about less and less. The need for accurate methods where but very small quantities of material are available for analysis is perhaps nowhere greater than in the field of public health chemistry. In air analysis, dust analysis, in foods and many other materials where minute amounts of contaminating substances are sought, micro methods are continually being developed. The field in which Pregl is authoritatively supreme is that of organic microanalysis.

The demand for authoritative methods of organic microanalysis is shown by the fact that Pregl's "Quantitative Organic Microanalysis" has passed through three German and two English editions within a relatively short time. The development in microchemistry has been such that a new journal exclusively devoted to this field, *Die Mikrochemie*, has been founded.

The treatment throughout Pregl's manual anticipates the needs of the beginner. The book in the first place is very readable. Not only are the reasons given for each step, but a more or less historical introduction is given to the more important topics. The discussion is very thorough and is amplified by means of clear-cut drawings.

In addition to the operations of ultimate organic analysis discussed in earlier editions, the author has added sections dealing with the determination of the halogens in the presence of one another, the acetyl group, molecular weight by Rast's melting point method, and measurement of the specific rotation of optically active compounds. The procedures dealing with the microdetermination of phosphorus, sulphur, arsenic, mercury, and copper are of particular interest to the public health chemist concerned with the microdetermination of these elements.

While the author is somewhat skeptical (p. 230) of the average chemist's ability to acquire the manipulative dexterity and technic required for this type of microchemical analysis without personal tuition, the very full and authoritative exposition of this manual will be found to be an invaluable introduction to the subject.—*L. T. Fairhall.*

J. I. H.
March, 1931